On Ganymedes anaspidis (nov. gen., nov. sp.), a Gregarine from the digestive tract of Anaspides tasmaniæ (Thompson).

By

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With Plate 11, and 5 text-figures.

Introduction.

In 1907 Mr. Geoffrey Smith was in Tasmania on a zoological errand, his object being especially to investigate the structure and development of Anaspides, the Mountain Shrimp of that country.

After his return to England, he noticed, while examining his sections in detail, some curious structures in the liver, which on investigation proved to be large binucleate cells, obviously of parasitic origin. Turning his attention to the gut, he found that it was in some cases inhabited by large numbers of Gregarines of an unusual type, and surmised that there was a connection between these and the non-motile parasites in the liver.

This was enough to show that Anaspides, so interesting in every detail of its structure, is no less so in regard to its parasites; and, as he had much work of his own on hand, he kindly offered me the congenial task of describing this new Sporozoan, at the same time providing me with all his surplus specimens of Anaspides. For this, and for much help and advice, I must here tender my best thanks; nor must I

forget to express my gratitude to Prof. Bourne for much kind assistance.

Methods, etc.

Preservation.—Some of the Anaspides had been pickled in formalin, some in corrosive sublimate; these latter were much better preserved, and were exclusively used in the work.

Preparation of the Gregarines.—Mr. Smith's specimens of Anaspides had been kept in captivity for some time before they were preserved; and, either they had had very little to eat, or else all the fare provided for them was digestible—at all events their guts were almost empty, save of parasites. Thus it was easy to make preparations of large numbers of the Gregarines by staining the gut and liver-tubes whole in paracarmine for a couple of hours, and then, after taking up to xylol, teasing in Canada balsam on the slide, and removing as much of the débris of the gut as possible, leaving the parasites behind.

This was quite good for general features, but, as I found to my cost later, did not bring out certain important cytoplasmic structures.

Subsequently some more Anaspides were sent over from Tasmania; these had been preserved at the moment of capture, and their guts were filled with a mass of sand, swallowed for the sake of the contained organic fragments. This made matters more difficult. The Ganymedes had to be picked one by one out of the débris by means of a capillary pipette under the binocular microscope. They were then mounted from 90 per cent. alcohol on to a film of egg-albumen smeared over a slide, so that they could be stained with Heidenhain's iron hæmatoxylin, which proved much the best reagent for picking out the details of the complicated structures in the cytoplasm.

Besides making these whole preparations, I had sections

cut of individual parasites, and of the gut and liver of the host. Most of these were stained with iron hæmatoxylin, some with Ehrlich's hæmatoxylin and eosin, and some with methyl-blue eosin by Mann's method. Iron hæmatoxylin was the best for most purposes, but Mann's method was very interesting in revealing some of the complexity of the purely vegetative processes that take place in the nucleus and nucleolus.

It was of course impossible to get any Anaspides over to England alive, and thus several questions of structure and life-history which could probably have been easily elucidated by observations and cultures of the living Gregarine, have had to be left to await the verdict of some investigator who has not got the Tropics between himself and the source of his material.

General Account (Life-history, Habitat, Etc.).

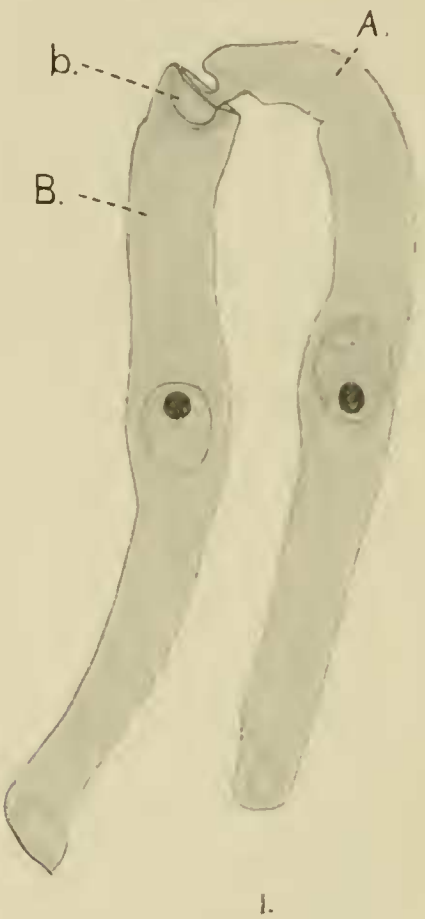
As above mentioned, Ganymedes is a parasite of the Syncaridan Crustacean Anaspides tasmaniæ, inhabiting various portions of its digestive tract. Before proceeding to a detailed account of its structure, it will be best here to give a brief general survey of its life-history, as far as such a continuous record can be pieced together from the mere snap-shots which are all that preserved material can give.

I came across no sporozoite stage. The smallest Gregarines found were only about one-eighth the length of the full-grown motile trophozoite, but otherwise similar in every way. These elongated motile forms, obviously belonging to the class Gregarinida, are in what I shall call the first trophic period, which is spent within the very long mid-gut of the host. Here some are attached to the epithelium (fig. 9), but the majority are found free in the lumen. If the host has recently been feeding, the gut is crammed with sand-grains and organic particles; and when this is the case, the parasites collect between this food-mass and the gut-wall,

where there is plenty of food that they can absorb, and least chance of their being carried away to the exterior.

Sometimes the parasites, instead of having their typical straight or gently-curved form (fig. 1) lie coiled and con-

TEXT-FIG. 1.



1.

An associated couple of Ganymedes, showing the cup-individual (B) grasping in its cup the ball (*b*) of the other associate (A) The ball end of B is abnormal. The cup end of A has a large vacuole within it. The bodies are slightly dilated round the nucleus.

torted against the intestinal wall; and when this is so, many are usually congregated in patches, and are stuck together, presumably by the coagulated secretion of the endodermic cells. What are the reasons for this condition I could not discover.

Finally, a certain number of the Gregarines are found associated in pairs, the attachment being by dissimilar ends (text-fig. 1). Not very many are in this state, but I suspect that the shock of killing, and the subsequent manipulation, manage to sever the connection between a large number of couples, and therefore cannot say if association always supervenes when the parasites reach a certain size, nor what are the proportion of couples to free Gregarines.

Association marks the close of the first trophic period. In the second trophic period the Gregarines are non-motile, have lost all the complex structure they had before, and are characterised by their (probably rapid) growth to a very large size. In this state they are found in the liver-tubes, of which there are twenty or thirty, lying free in the hæmococœle, and not intertwined. It follows that the associated couples must migrate forwards to the junction of mid- and fore-gut, where the liver tubes open, and thence back into one of these. On penetrating a safe distance along the tube, a transformation must take place, the two Gregarines undergoing complete cytoplasmic fusion, a state of affairs known hitherto only in those neogamous forms from Holothurians, Cystobia and allied genera (Woodcock, 6).

These fused couples, looking just like one cell with two nuclei, are found wedged in between the cells of the wall, with a considerable free surface for absorption towards the lumen of the tube. There is often another free surface on the exterior, due, I should say, simply to the growth of the creature, and the consequent forcing apart of the liver-cells (text-fig. 3). For this growth, Ganymedes is here in a very favourable place, since the so-called liver, in addition to producing digestive ferments, is the organ where a great part of the food is absorbed; and so, while the parasites may enter on this period when measuring no more than $70 \times 60\ \mu$, they often attain to the considerable size of $200 \times 130\ \mu$, and I have seen one that measured $300\ \mu$ in its greatest length, though its breadth was only $120\ \mu$. The shape is variable, from a nearly perfect sphere to a long ellipsoid or ovoid.

The two nuclei meanwhile become round and very large, and possess on one side a large lenticular nucleolus.

The next step in the cycle is for the associated couple, while still in the liver-tube, to form a thick resistant coat round itself: in so doing it becomes perfectly spherical, and a process of concentration of cytoplasmic materials must take place, as I have found none of these cysts with a diameter of more than 115 μ, and one only 85 μ across, the average being about 100 μ.

The formation of the cyst wall of necessity closes the trophic periods, and sporogony now presumably begins. I say presumably, for I have seen no spores, nor even any of the preparatory nuclear divisions. Two cysts in the liver of a particular host showed nuclei with central nucleoli emitting chromatin (fig. 17)—a phenomenon very common in Protozoa at the close of vegetative life: and I have found a number of the usual type of cysts free in the gut.

From these facts, and from analogy with other intestinal Gregarines, we must suppose that after the formation of the smooth cyst wall, the couples can be expelled from the liver tubes (while those in the second trophic period remain in place by virtue of their soft surface adhering to the similar surfaces of the liver-cells), that they are then passed out by the anus, and that it is only here, under the stimulus of the changed conditions, that the processes leading to the production of spores can take place.

This being so, it is probable that infection is casual, the spores or sporocysts being taken in with the food—as, indeed, might have been deduced from the feeding habits of Anaspides. The infection is usually heavy (text-fig. 3), and frequently seems to be multiple, cysts, motile Gregarines, and associated immobile forms being often found all in one host. The proportion of infected hosts was over 50 per cent. in the case of those that were captured by Mr. Smith in a small pool on one of the mountain becks of Mt. Wellington; but in those he obtained from a larger piece of water, the infection was nil—or at least no parasites were forthcoming

in the dozen or so of hosts that I examined. The time of year seems to have no effect on any of the processes of the parasites' life.

As regards the effects produced by Ganymedes, no inconvenience seems to be suffered by the organism of the host as a whole, and only trifling damage is done to individual tissues. Those few cells of the gut epithelium to which the Gregarines attach themselves look generally unhealthy, and their nucleus becomes hyperchromatic (fig. 10); and the walls of the liver tubes get more or less distorted by the growth of the large couples in the second trophic phase: but in neither of these ways can any serious harm be done.

After these preliminary remarks, we may now proceed to consider in detail the structure of Ganymedes in its various stages.

Detailed Account.

(i) The First Trophic Period.

Although the size of the smallest free Gregarines seen was only 80—100 μ, yet I could find no points of difference between them and the adults, save that in the young forms the body has not attained to its full size relative to the structures (soon to be described) situated at the extremities. From these small forms all stages may be seen to Gregarines 400—425 μ long, and 23—30 μ broad, though the average size is 250—300 μ × 17—20 μ.

The shape of the body is cylindrical, tapering slightly towards one end, and considerably towards the other. The thinner end is almost certainly anterior in progression, and when attachment takes place, it is by means of a structure at this extremity. This structure in favourable specimens is seen to consist of a sphere connected by a thinner neck to the main body: I propose to call it the ball, and the thin extremity on which it is placed, the ball end. The other end may be called the cup end, for here many individuals possess a perfectly regular hemispherical depression, whose

outside walls continue the lines of the body: the whole is marked off by a circular groove, thus rather resembling the sucker of an Octopus.

Leaving the details of these organellæ for the present, I will now describe the main body of Ganymedes. This is of the usual type seen in motile Gregarines. It is covered with a firm cuticle, the longitudinal striations on which can be easily seen (figs. 6, 10, 11). Just beneath this appears in many cases a pale ectoplasmic layer, lacking the granules of the central endoplasm: and though I have never been able to demonstrate actual myonemes, yet from what we know of other Gregarines it is probable that this layer is the seat of the contractile structures which this free-swimming creature must possess. The endoplasm proper is denser, and contains granules. The whole cytoplasm is of reticular or alveolar structure.

The nucleus lies more or less in the centre of the body: it is ellipsoidal: the folds and processes sometimes seen at one end of it (fig. 15) being probably artefacts. Its breadth is often very nearly that of the Gregarine, and it would sometimes touch the cuticle except that when it is large the body bulges out slightly round it. It possesses a thin but distinct nuclear membrane, within which is a reticulum with granules on the threads—sometimes loose with largish grains (fig. 14), sometimes finer (fig. 15). In addition there is present a deeply staining spherical nucleolus, usually towards the cup end of the nucleus. In it, a thin outer rind usually stains deeper than the central medulla, which is filled with clear vacuoles of various sizes (figs. 14, 15). With Mann's methyl-blue-eosin it stains usually bright crimson to claret-colour, often with a violet crescentic area on one side.

Returning now to the anterior extremity, we find that in some cases there is, as above stated, a distinct stalked sphere (figs. 7—10). This is covered with a cuticle thinner and less firm than that of the body, the two passing into each other round the narrowest part of the neck (fig. 7). The sphere is filled with a quite homogeneous fluid, except at the extreme

front end, where there is usually a sort of pad of fine-grained cytoplasm projecting back into the cavity (fig. 8). In the main body, behind the neck, is another spherical cavity, apparently separated from that of the ball proper, and containing a fluid that is not quite clear, but of a loose reticulate structure (figs. 6, 7). Enclosing the hinder part of these may sometimes be seen a dark crescent of nearly homogeneous material (fig. 9).

So far, so good. In other cases, however, we find quite a different appearance, there being only one cavity present, and all traces of a neck having vanished (figs. 3, 5). Closer inspection shows that the cavity corresponds with that of the true ball, as its contents are perfectly clear, and it has a pad of cytoplasm anteriorly. The dark crescent may come directly behind it (fig. 5), while the thick body cuticle extends completely over it. The question then is, what is the relation between these two conditions?

It seems obvious that the ball can be extruded at will —but in what way? Is it evaginated (pleurecbolic) or is it acrecbolic, and, if the latter, is it pulled out by muscular or elastic action or pushed out by some other means; and how is it retracted? An examination of many Gregarines (a task necessary owing to the absence of living material, but laborious from the small size of the ball— 8—10 μ across), has made it seem probable that it is acrecbolic, and pushed out by the accumulation of a watery fluid behind it. As far as I can make out, the structures and processes concerned are as follows:—The dark crescent (*s.t.* in figs.) is a tissue which has the power of secreting a fluid (*w.*) into a space anterior to it, thus driving the ball out through an opening in the body cuticle. When the ball is retracted, the elastic cuticle would be closed over the anterior end; and when extrusion has taken place, it would press in and form the thin neck. One animal (fig. 2) shows what I suppose to be an early stage of extrusion: the hole is just being enlarged, so that the cuticle at its edge stands out as a well-marked rim (*cut. rim*). In later stages (figs. 4 and 6)

this rim will press against the convexity of the ball and thus be difficult to see; it is only in the early stages of extrusion that its inner surface will form an angle with the surface of the ball, and thus stand out. The pad of cytoplasm (*p.*) is always seen at the anterior end of the ball vesicle, showing that there can be no question of invagination.

Retraction would then take place by the resorption of the secretion; while the ball seems to be kept in place by strands from the ectoplasm (probable muscular layer), for this, and this only, usually extends up the sides of the secreted fluid to the ball vesicle (figs. 3, 5, 6).

When fixation takes place, the condition of things looks somewhat different (fig. 10), and there is an open communication from the ball to the space behind it. Very possibly the cytoplasm at the neck is temporarily dissolved so as to leave this passage-way for the food absorbed by the ball to pass further into the substance of the animal.

Finally, in association, the ball of one is extruded into the cup of the other, and the cup then seemingly contracts so as to hold the ball firm (fig. 9; text-fig. 1). It may be here remarked that the free ball end in the couple in text-fig. 1 is quite abnormal: it was pointed, and contained a pointed cavity within it, but otherwise had none of the typical structure.

The cup-end also presents various difficulties. When well formed its structure is simple enough, and has already been described. But at other times the hollow cup may be quite wanting, the body ending simply in a rounded end with rather thick ectoplasm (fig. 12); or, more often, there are numerous vacuoles beneath the cuticle (fig. 13), with sometimes an irregular aperture in addition (text-fig. 2). What the meaning of these variations is, and whether the cup-end can pass from one state to another, I fear I cannot say.

It was from the presence of the cup that I ventured to call this new genus Ganymedes, though the pedant will perhaps maintain that this name should have been reserved for some

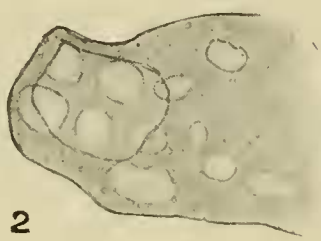
parasite of Aquila. With the specific title anaspidis, however, I think no one will quarrel.

(ii) Second Trophic Phase.

Between the two phases of trophic life no intermediate stages were found, all the couples in the liver having lost every trace of the cytoplasmic structures of the Gregarinoid form. All they possess is a thin cuticle (fig. 18), investing a delicately-meshed cytoplasm.

The nucleus, on the other hand, has increased in complexity (fig. 18). It is large and more or less spherical,

TEXT-FIG. 2.



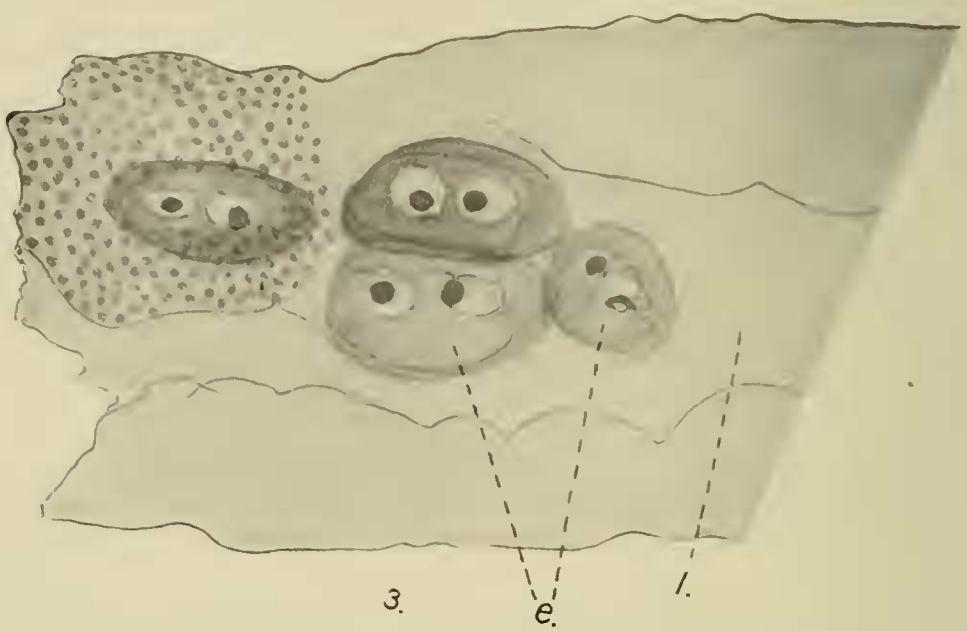
Diagrammatic view of the cup end of a Gregarine, to show the opening on one side, and the numerous vacuolar spaces in the cytoplasm.

with a thin nuclear membrane, and an achromatic network in which there is very little chromatin present. The chief interest lies in the nucleolus, which is peculiar in two ways. First, it occupies an unusual position, right on one side of the nucleus, somewhat like the lens of an eye, with a considerable surface in contact with the cytoplasm—a state of things not, I believe, known in any other Gregarine, though Awerinzew (1) has described something similar for a Myxosporidian; and secondly, it possesses itself another lens-like structure, projecting more or less into the cell-body, and composed of a very pale-staining meshwork, with its outer border not a smooth curve, but formed of the slightly projecting parts of the component alveoli (fig. 18).

This is perhaps the absorptive part of the nucleolus, taking up from the cytoplasm the soluble food which this in its turn has abstracted from the liver-tubes.

In the centre of the nucleolus, abutting on the absorptive part, is often an area, with a reticular structure, staining blue-violet with Mann's method. The remainder is composed of a dense material staining deep red, in which are embedded definite clear pink vacuoles. Towards the cyto-

Text-fig. 3.



Portion of a liver-tube of Anaspides with four couples of Ganymedes in it. The nuclei of the liver cells are represented only in one corner. *l* = lumen of liver tube. The lighter parts of the parasites (*e*) are exposed on the exterior of the liver-tube.

plasm these vacuoles project slightly; when one sticks right out, as at *x*, fig. 18 *b*, it is colourless, showing that the others look pink only because there is red substance above and below them. Towards the nucleus, on the other hand, the vacuoles rarely project, the edge of the nucleolus being usually clean cut. Text-fig. 4 represents diagrammatically another nucleolus in which the absorptive area was extremely large.

The nucleolus thus seems obviously to be the chief agent concerned in the manufacture of food-stuffs (for theories regarding the action of Mann's methyl blue eosin see Léger and Duboscq (2)).

What is the function of the rest of the nucleus in this period remains uncertain, though its large size shows that it must play some important part in metabolism. The chief interest here lies in the behaviour of the nucleolus, which migrates out to enter into direct relations with the cytoplasm at the beginning of the second trophic period, when assimilation begins to be greatest, and at its close, when all

Text-fig. 4.

Section of one of the nuclei of a couple in the second trophic phase. The nucleolus does not project very far, and the surface of the absorptive area is flush with that of the nucleolus, although the area itself is very large.

assimilation ceases, returns, as will be seen later, to the interior of the nucleus.

(iii) Encysted Phase.

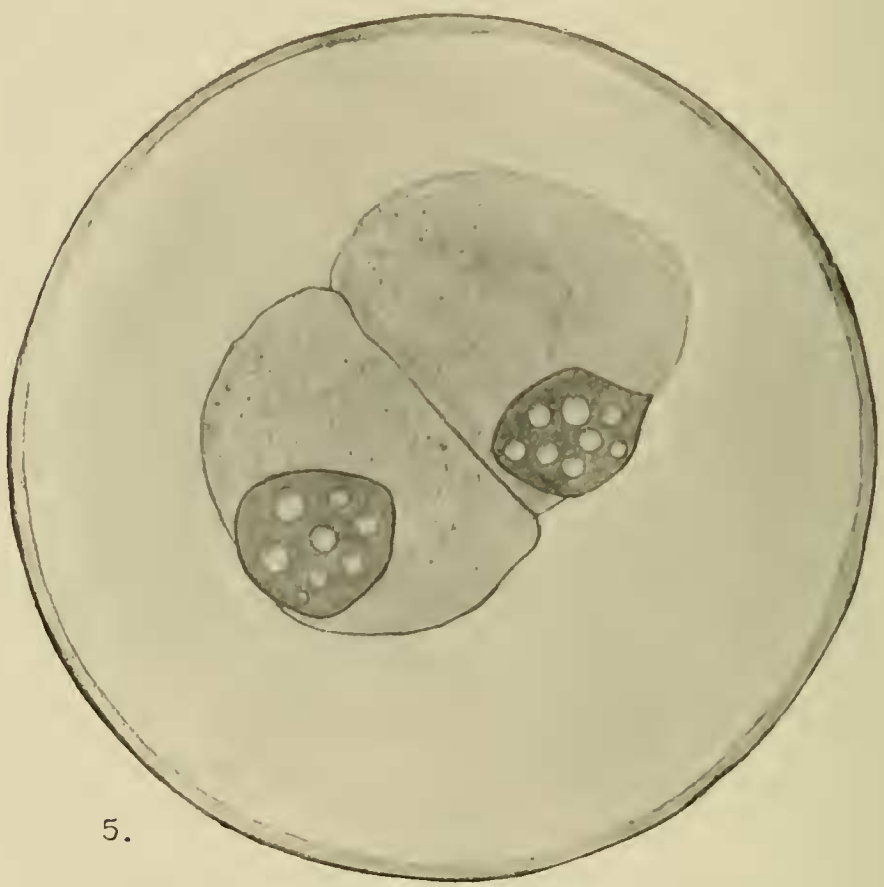
The cyst-wall, though always fairly strong, varies a good deal in thickness. It stains bright blue by Mann's method, bright red with carmine, but not strongly with hæmatoxylin. From it often project radially inwards curious irregular, branching filaments, never reaching much more than a third of the way to the centre, as to whose nature and function I am quite in the dark (fig. 16).

The cytoplasm is reticular, with minute granules on the

threads, and larger, chromatic granules here and there. It always looks denser than in the unencysted forms.

The nuclei in what I take to be the earlier cysts are much like those described for the second trophic phase, except that they stain a little deeper, and that the nucleoli do not project so far out from the surface (text-fig. 5). In the next

TEXT-FIG. 5.



A cyst found in the gut. The nuclei are not actually touching, but very near to each other. The cyst-wall is very thick in this specimen.

stage (fig. 16) the nuclei, bounded only by a very thin membrane, stain quite deeply, as they are almost filled with chromatic granules of various sizes. The nucleolus is still in contact with the cytoplasm, but its outer surface is now flush with that of the nucleus. This outer border of the nucleolus is made up of rows of minute vacuoles, while the

rest is dense, with a clean-drawn boundary towards the interior, and homogeneous except for a few large vacuoles.

To this stage probably belongs the cyst in fig. 19, stained by Mann's method. The nucleolus is blue, having given up most of its chromatin to the nucleus, which is violet with dark purple grains.

In fig. 17 we have another state of affairs: The nucleolus, now retreated from the surface, seems to be giving off chromatin to the nucleus in the shape of hollow spherules. It is itself formed of a single central vacuole, surrounded by a layer of small ones embedded in a dense chromatic cortex (the lower nucleolus is cut tangentially, and so does not show this condition). The nucleus, apart from the chromatic spherules, appears perfectly homogeneous, with no achromatic network, and differs also from the nuclei of other stages in being amœbiform, with "pseudopodia" that can be very clearly seen on focussing up and down.

From what we know of other Gregarines, it is clear that these stages are preliminary to the breakdown of the large trophic nuclear apparatus, and the reconstitution of the idiochromatin to form the gametocyte nucleus. But, as above mentioned, the cysts soon after this pass into the gut and out by the anus, so that their further development must remain for the present unknown.

Conclusions: Systematic Position.

Though here more than ever must we lament the absence of spores, it is still possible to draw some fairly definite conclusions. To start with, Ganymedes is not a Polycystid, nor does it belong to any existing family among the Monocystids. Thus a new family, the Ganymedidæ, must be created, whose characters will provisionally be those of the genus: these may be here conveniently summarised as follows:

(1) The possession by the motile form of a special extensible organ at the front end, which may serve for fixation to the cells of the host.

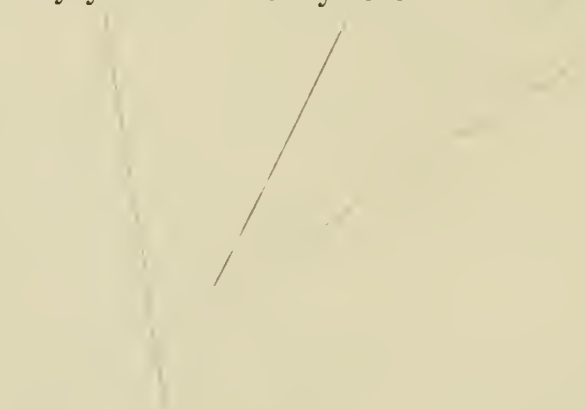
(2) The presence of a special cup-like structure at the posterior end, which co-operates with the epimeritic organ at the anterior end to effect a close union of two individuals during association. Association is thus by dissimilar ends, and lasts for some time.

(3) The eventual complete cytoplasmic fusion of the associated couples, and the existence of a second trophic phase, when the animals grow very fast, but are morphologically quite degenerate.

(4) The position of the nucleolus in this phase, on one side of the nucleus, partly in contact with the cytoplasm.

(5) The habitat, in the gut and liver of Syncaridan Crustacea.

Considering these characters in relation with other members of the class, we find that no known Gregarines inhabit the liver of any Crustacean; none have the nucleolus in the same position; none go through two trophic phases; none have any special structure for association at the posterior end; and none have a protrusible organ of the same sort at the front end. It is thus at least obvious that Ganymedes is the representative of a very divergent line. The suggestion I would make is that, while nearer to the Monocystid type, Ganymedes is partly intermediate between the two great groups of Eugregarines, as represented diagrammatically in the following tree:



In the first place, the ball and the cavity containing the secreted fluid represent with great probability an epimerite and protomerite. True, there is no cuticular septum; but the secreting tissue forms a fairly definite barrier between these on one side, and on the other the deutomeritic posterior part. Here alone, it is to be remarked, do we find the true granular endoplasm. Occasionally, too, this latter can be seen ending off with a definite contour within the secreting tissue (fig. 3). The ball itself, when extruded, would pass for a typical epimerite save for the absence of a septum behind it; but in so far as it is protrusible, it is only paralleled by the anterior extremity of Lankesteria ascidiæ (Siedlecki, 4). This, however, seems to be merely a pseudopodium, or a drop of the hyaline inter-reticular substance of the cytoplasm pressed out through a hole by contraction of the animal, and its extrusibility has obviously been independently evolved.

The fact of its being a parasite of the digestive tract is the second link with the Polycystidea. The only Monocystid gut-parasite whose life-history has been thoroughly worked out is Lankesteria, and this possesses an "epimeritic" organ. The three or four other genera of this sub-class that live in the gut, such as Callyntrochlamys and Ancora, are very insufficiently known; it is even possible that they may be Polycystid in early stages.

Regarding the matter phylogenetically, we find that the early Eugregarine stock must have been motile, Polycystid gut-parasites; their association was by dissimilar ends, and took place only at the very end of the trophic period; and they showed well-marked anisogamy.

One of the first steps towards the typical Monocystid condition was the change of habitat, due very likely in the first instance to the evagination of the full-grown trophozoites from the gut into the cœlom—as takes place to-day in certain insect-parasites at the time of the host's metamorphosis. For a full discussion of the further stages, leading eventually to complete isogamy, coupled with entirely

cœlomic habitat, precocious association, and degenerate structure, the reader is referred to Woodcock (**6**). Suffice it here to say that the course of affairs in Ganymedes must have been somewhat different. It is probable that Ganymedes at first associated only at the close of the trophozoite stage. Some of the couples having migrated into the liver, found it (like the cœlom for other Monocystidea) a safe retreat and abounding in soluble food. Here too the Gregarine could afford to dispense with all the structures necessary for a life in the open gut, and devote all its energies to growing. One might have thought then that Ganymedes would have associated in the sporozoite stage, like Cystobia, and migrated at once into the liver; but, whether non-motile couples below a certain size could be expelled from the tubes or be engulfed and digested by the activity of the liver-cells (see Smith, **5**, p. 536), or from some other cause, Ganymedes has found it necessary to remain in the gut till it has attained a definite bulk, thus presenting to us the phenomenon of two sharply-distinct trophic phases after the sporozoite stage. As the parasites are non-motile when they are about to sporulate, conjugation must needs be precocious, so that no Gregarine shall migrate alone into the liver, and thus be, from the point of view of the species, wasted. For this fairly lasting association some special mechanism was imperative, hence the cup and ball; while the necessity of remaining some time in the gut has led to Ganymedes retaining more of the original Polycystid structures than is usual in the morphologically degenerate Monocystidea. Finally, although the sporogony remains unknown, it may be confidently prophesied that this Gregarine will be found to be completely isogamous.

Thus it will be seen that the Ganymedidæ diverged very early from the Monocystid stock, and possess now many new and peculiar characters intermixed with those they have inherited from the common ancestor. For the complete disentangling of these from each other, further work must be done on Ganymedes, and in addition all

Syncaridan Crustacea should be searched for allied parasites, whose structure would at once give us new standpoints from whence to view the problem.

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EXPLANATION OF PLATE 11,

Illustrating Mr. Julian Huxley's paper "On Ganymedes anaspidis (nov. gen., nov. sp.)."

Reference Letters for the Figures.

b. Ball-cavity. *c. s.* Cuticular striæ. *ect.* Ectoplasm (probable myocyte layer). *p.* Cytoplasmic pad at anterior end of ball. *s. t.* Secreting tissue. *v.* Vacuoles. *w.* Secreted fluid that accumulates to drive the ball out.

Bor.-car. Borax carmine. *Paracarm.* Paracarmine. *Hæm.* Hæmatoxylin. *M. B. E.* Methyl-blue eosin (Mann's method).

PLATE 11.

Fig. 1.—Large individual at the close of the first trophic stage, with well-formed cup. (Paracarm. × 640.)

Fig. 2.—Ball end of the same, to show the ball being pushed out through the hole in the cuticle; the edges of this hole stand out markedly as a rim (cut. rim). (× 1300.)

Figs. 3–8.—Ball ends of various Gregarines in different conditions.

Fig. 3.—(Semi-diagrammatic). Very slightly extended. Secreting tissue very large, with the granular endoplasm (e_1) ending off within it. Outside is a non-granular layer (e_2), and just beneath the cuticle the still paler ectoplasm, extending on the left to touch the ball-vesicle. (Bor. Carm. × 1875.)

Fig. 4.—Semi-extended. The secretion of the secreting tissue is fairly dense. The double contour of the hinder part of the ball is well seen. There seems to be no ectoplasm. (Iron Hæm. × 1875.)

Fig. 5.—(Semi-diagrammatic.) Completely retracted. Very large cytoplasmic pad (p) with dark grains in it. A large dark granule in the secreting tissue. The ectoplasm extends to touch the ball. (Iron Hæm. × 1875.)

Fig. 6.—Almost extended. The secreted fluid has here a wide-meshed structure. The thick body-cuticle ends abruptly where it touches the ball, which possesses only a thin cuticle. Cuticular striæ are seen on the under surface. No well-differentiated ectoplasm. (Iron Hæm. × 1875.)

Figs. 7 and 8.—(Semi-diagrammatic.) Completely extruded.

In fig. 7 the neck of the ball is well seen, also the more delicate nature of the ball's cuticle. No cytoplasmic pad is visible.

In fig. 8 the ball is directed slightly upwards. The cuticle is distended round the secreted fluid, showing that this is under pressure. (Paracarm., fig. 7 × 1300; fig. 8 × 1875.)

Fig. 9.—Section (5 μ) through the point of junction of an associated couple in the first trophic phase. The cytoplasm of the ball individual (A) is denser than that of the other (B). (M. B. E. × 1300.)

Fig. 10.—Section (5 μ) through the point of attachment of a mobile Ganymedes to a cell of the host's gut. The cuticular striæ are well seen. The ball is thrust into the host-cell, and contains a fluid that is not clear, the reticular structure being probably due to the coagulation of absorbed food. There is an open passage through the neck into a

cavity in the body of the parasite. The cytoplasm contains numerous deeply-staining granules. The nucleus of the host-cell (*n*) is large, darkly-stained, and homogeneous, except for some dark grains. (Iron hæm. × 1340).

Figs, 11–13.—(Semi-diagrammatic). Cup-ends.

Fig. 11.—Cup-end of the Gregarine whose ball-end is shown in fig. 5; (*a*) is focussed near the upper surface, and shows how the cup is separated from the body by a circular groove; (*b*) shows the greatest diameter of the cup. (Iron Hæm. × 1875.)

Fig. 12.—Cup-end of another Gregarine, to show absence of all differentiation. The ectoplasm is thicker at the end than elsewhere. (Paracarm. × 1300.)

Fig. 13.—Section of the cup-end of Gregarine A in fig. 9, to show the numerous vacuolar spaces beneath the cuticle. (M. B. E. × 1300.)

Figs. 14 and 15.—Sections (5 μ) to show the structure of the nucleus in the first trophic phase. (M. B. E. × 1300.)

Figs. 16 and 17.—Sections of cysts.

In fig. 16 the filamentous inward projections from the cyst-wall can be seen. Small chromatic granules fill up the nucleus; there is no sign of an achromatic network. The nucleoli are retreating to the interior of the nucleus. (Iron Hæm. 5 μ × 970.)

In fig. 17 the nuclei are amœboid, filled with a homogeneous sap in which are hollow chromatic spherules, apparently emanating from the nucleoli. The cyst-wall is crumpled, and in one place a flap of it has got detached so that its surface-structure is seen. (Ehrlich's hæm. + eosin 10 μ × 800.)

Fig. 18*a*.—Section (5 μ) through an associated couple in the second trophic phase. The reticular nature of the cytoplasm is not indicated. (M. B. E. × 610.)

Fig. 18*b*.—The next section in the series. The nucleolus and the outline of the nucleus are given, more highly magnified. The three areas of the nucleolus and their structures are shown (see text). At *x* a vacuole projects beyond the general surface, and is seen to be colourless. (M. B. E. × 870.)

Fig. 19.—Section of a cyst, to show the alteration in staining reactions of nucleus and nucleolus in this stage (see text). (M. B. E. × 400.)